İSTANBUL UNIVERSITY PRESS

Aquatic Sciences and Engineering

Aquatic Sciences and Engineering,
ASE 2025 , 40 (4): 278–284

https://doi.org/10.26650/ASE.2025.1757673
Submitted: 03.08.2025
Revision Requested: 24.09.2025
Last Revision Received: 25.09.2025
Accepted: 02.10.2025
Published Online 06.10.2025

Research Article

Open Access

Determination of Amylolytic and Proteolytic Enzyme Activity Characteristics of Thermophilic *Actinobacteria* Isolated from Amasya-Terziköy Thermal Spring

Demet Tatar [1], Aysel Veysioğlu [2], Ali Tokatlı [3,4] & Hünkar Avni Duyar [5]

[1] Hitit University, Osmancık Ömer Derindere Vocational School, Department of Medical Services and Techniques, Çorum, Türkiye
[2] Sinop University, Vocational School of Health Services, Department of Medical Services and Techniques, Sinop, Türkiye
[3] Technology Transfer Office, Çankırı Karatekin University, Çankırı, Türkiye
[4] Ondokuz Mayis University, Faculty of Science, Department of Biology, Samsun, Türkiye
[5] Sinop University, Fisheries Faculty, Department of Fishing and Processing Technology, Sinop, Türkiye

Abstract *Actinobacteria* represent a group of Gram-positive microorganisms characterized by high G+C content and are commonly found in both terrestrial and aquatic ecosystems. These bacteria are notable for their capacity to synthesize a wide range of bioactive compounds, including antibiotics and industrial enzymes. Their ability to withstand extreme environmental conditions allows them to colonize challenging habitats, such as thermal springs. These geothermal environments are critical not only for harboring microbial diversity but also for the discovery of novel bioactive substances with therapeutic potential. This study focused on isolating thermophilic actinobacteria from Terziköy Thermal Spring in Amasya, Türkiye, followed by phylogenetic analysis through 16S rRNA gene sequencing. The enzymatic capacities of the isolates—specifically amylase and protease production—were also assessed. Fourteen of the 21 isolates underwent 16S rRNA sequencing, resulting in the identification of eight *Streptomyces*, four *Nocardia*, one *Actinomadura*, and one *Pseudonocardia* species. Among these, seven isolates demonstrated amylase activity, and one displayed protease production. Notably, one *Streptomyces* isolate exhibited both enzymatic activities. These results highlight the phylogenetic diversity of thermophilic actinobacteria in geothermal environments and their potential as sources of thermostable enzymes for industrial applications.

Keywords Thermophilic bacteria · 16S rRNA gene · amylase and protease activities

Citation: Tatar, D., Veysioğlu, A., Tokatlı, A. & Duyar, H. A. (2025). Determination of Amylolytic and Proteolytic Enzyme Activity Characteristics of Thermophilic *Actinobacteria* Isolated from Amasya-Terziköy Thermal Spring. *Aquat Sci Eng*, *40*(4), 278-284. https://doi.org/10.26650/ASE.2025.1757673

Corresponding author: Demet Tatar demettatar@hitit.edu.tr

INTRODUCTION

Actinobacteria are a diverse group of Gram-positive bacteria widely recognized for their significant contributions to biotechnology and pharmaceutical industries, particularly due to their ability to produce a broad spectrum of biologically active molecules. These microorganisms are responsible for generating approximately two-thirds of antibiotics derived from microbial sources (Challis & Hopwood, 2003), and more than 70% of therapeutically relevant bioactive compounds used in medicine originate from actinobacteria (Bérdy, 2005).

In recent years, growing attention has been directed toward thermophilic and heat-tolerant actinobacteria because of their potential to produce both primary and secondary metabolites with applications in medicine, agriculture, and biotechnology (Tang et al., 2006; Elbendary et al., 2018). Recent reviews further emphasize the exceptional potential of thermophiles and extremophiles as sources of novel enzymes ("extremozymes"), which are valued for their stability and functionality under industrially relevant conditions (Shomali & Danish-Daniel, 2024; Gorrab et al., 2024).

These bacteria are predominantly free-living and have been recovered from a variety of ecosystems including freshwater and marine environments (Ma et al., 2009; Veyisoglu & Tatar, 2021), soil habitats (Elbendary et al., 2018), island environments (Veyisoglu et al., 2024), deep-sea locations (Pathom-Aree et al., 2006), polar regions like Antarctica (Rego et al., 2019), and arid desert soils (Busarakam et al., 2016). Although they exist in various soil layers, their population density tends to decline with increasing soil depth (Takahashi & Omura, 2003). Beyond their ecological versatility, some actinobacteria are known to be pathogenic, with the ability to cause diseases in humans (Könönen & Wade, 2015), animals (Ertaş et al., 2005), and plants (Lerat, 2009).

Research focusing on actinobacterial biodiversity in thermal environments remains limited, and findings have varied. For instance, some studies report the absence of actinobacteria in highly acidic and volcanically influenced hot springs (Goodfellow & Williams, 1983), while others have found only sparse populations (Lacey, 1988).

Although hot springs typically contain minimal organic material, they are rich in various ions such as sodium, calcium, chlorides, bicarbonates, and sulfates. This unique ionic composition, combined with elevated temperatures, creates an extreme yet distinctive microbial habitat. In such oligotrophic conditions, actinobacteria can display exceptional metabolic adaptability (Medjemadj et al., 2020).

Thermophilic and alkaliphilic actinobacteria are of particular interest due to their ability to produce a wide array of commercially important enzymes such as amylases, proteases, lipases, cellulases, xylanases, inulinases, dextranases, and keratinases. These enzymes serve numerous roles across diverse industrial applications. Amylase, for example, facilitates the hydrolysis of starch into oligosaccharides and simpler sugars like glucose and maltose, making it essential in food and syrup production. Proteases, accounting for more than 65% of the global enzyme market, are especially valued due to their wide applicability in industries ranging from food processing to detergents (Shivlata & Satyanarayana, 2015).

Terziköy Thermal Spring, located 36 kilometers from the center of Amasya, maintains a water temperature of approximately 39.5°C. Historically used since Roman times, it is reputed to aid in the treatment of rheumatism, gastrointestinal conditions, urinary tract infections, and nutritional deficiencies (Amasya İl Kültür ve Turizm Müdürlüğü, 2025).

The principal objective of this study is to isolate thermophilic actinomycetes from the Terziköy Hot Spring in Amasya, Türkiye, and to determine their taxonomic positions using 16S rRNA gene analysis. Phylogenetic dendrograms were constructed to examine evolutionary relationships. Additionally, the study aimed to identify isolates with potential to be novel species and to evaluate their amylolytic and proteolytic enzyme production capacities.

MATERIAL AND METHODS

Collection and Storage of Water and Soil Samples

One soil and one water sample were obtained from distinct points within the Terziköy Thermal Spring area. Coordinates for the sampling locations were recorded using an eXplorist 100 GPS device (Table 1). Upon collection, samples were transported to the laboratory under chilled conditions and stored at 4°C until further processing.

Table 1.
Location and Geographical Coordinates of Water and Soil Samples

Locality	Geographical Coordinates
T1-Water	40°28'22.3"N
	35°42'16.6"E
T1-Soil	40°28'22.7"N
	35°42'16.6"E

Isolation of Actinobacteria

To selectively isolate *Actinobacteria*, three culture media were employed: Starch Casein Agar-SCA (Küster & Williams, 1964), Modified Bennett's Agar-MBA (Jones, 1949), and Malt Extract-Yeast Extract-ISP2 medium (Shirling & Gottlieb, 1966), each supplemented with nystatin (25 µg/ml) and nalidixic acid (10

µg/ml). Formulations are detailed in Table 2.The isolation procedure followed the serial dilution technique. One gram of soil and 1 ml of water were each added to sterile Ringer's solution (9 ml) along with sterile glass beads and gently agitated for 45 minutes to ensure adequate dispersion of microorganisms. To suppress vegetative contaminants, the resulting 10^{-1} dilutions were heat-treated in a 55°C water bath for 30 minutes. These were then vortexed, and successive 10^{-2}, 10^{-3}, and 10^{-4} dilutions were prepared under aseptic conditions using sterile Ringer's solution.

Table 2.

Media Used for Selective Isolation of Actinobacteria

	Media to be used	Antibiotic
1	Starch Casein Agar (Küster & Williams, 1964)	Nystatin (25 µg/ml), Nalidixic acid (10 µg/ml)
2	Modified Bennett's Agar (Jones, 1949)	Nystatin (25 µg/ml), Nalidixic acid (10 µg/ml)
3	Yeast Extract-Malt Extract Agar-ISP 2 (Shirling & Gottlieb, 1966)	Nystatin (25 µg/ml), Nalidixic acid (10 µg/ml)

From each dilution, 0.2 ml aliquots were spread onto the surface of the selective media using sterile swabs. Duplicate plates were prepared for each dilution and incubated at four different temperatures (45°C, 50°C, 55°C, and 60°C) for 25–30 days to promote the growth of thermophilic actinobacteria.

Genomic DNA Isolation and 16S rRNA Gene Region Amplification

Genomic DNA was isolated from selected isolates using the Invitrogen PureLink Genomic DNA Mini Kit, following the manufacturer's protocol. Amplification of the 16S rRNA gene region was carried out using universal bacterial primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3'). PCR product integrity was confirmed via electrophoresis on 1.5% agarose gel stained with a nucleic acid dye.

Sequencing of PCR Amplified 16S rRNA Gene Regions, Sequence Data Analysis and Phylogenetic Dendrogram Construction

The amplified 16S rRNA fragments were sequenced by Macrogen Inc. (South Korea) using two forward primers and one reverse primer (Table 3). The resulting sequence chromatograms were assembled and edited using ChromasPro 1.7.5 software. FASTA-formatted sequences were compared to reference databases using the EzBioCloud platform (https://www.ezbiocloud.net; Yoon et al., 2017) to determine phylogenetic similarity to known taxa.

Table 3.

Primers Used in Sequencing Studies

Primer code	Sequence (5'-3')	Size	Reference
518F	CCAGCAGCCGCGGTAAT	17	Buchholz-Cleven et al., 1997
MG5F	AAACTCAAAGGAATTGACGG	20	Chun, 1995
800R	TACCAGGGTATCTAATCC	18	Chun, 1995

Multiple sequence alignments were performed using CLUSTAL W in MEGA version 12.0 (Kumar et al., 2024). Phylogenetic relationships were inferred using the Neighbor-Joining algorithm (Saitou & Nei, 1987) with evolutionary distances calculated by the Jukes-Cantor model. The robustness of tree topologies was tested using bootstrap analysis with 1000 replications (Felsenstein, 1985).

Examination of Amylase Production Ability

Amylase activity was detected by cultivating isolates on starch agar medium (composed of 1% starch, 0.3% NaCl, 0.1% KH_2PO_4, and 2% agar) for 7 days. After incubation, 1% Lugol's iodine solution was added to each plate. Hydrolyzed starch regions appeared as clear halos surrounding the colonies against a darkly stained background, indicating enzyme activity. Zone diameters were measured to quantify activity (Fossi et al., 2009).

Examination of Protease Production Ability

The ability to produce protease was determined by growing isolates on agar medium containing 1% casein, 0.5% yeast extract, and 2% agar. Following a 7-day incubation period, the formation of transparent zones around the colonies was recorded as an indicator of casein hydrolysis and proteolytic activity (Mohamedin, 1999).

RESULTS AND DISCUSSION

Selective Isolation of *Actinobacteria* from Water and Soil Samples

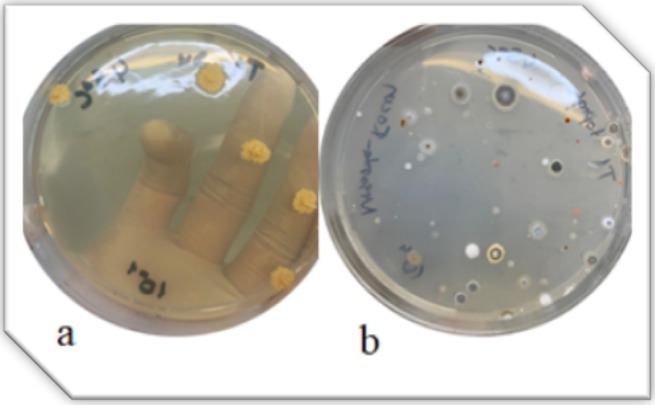
Aliquots (0.2 ml) from 10^{-1} to 10^{-4} serial dilutions of both soil and water samples were inoculated onto SCA, MBA, and ISP2 media (Shirling & Gottlieb, 1966), each supplemented with nystatin (25 µg/ml) and nalidixic acid (10 µg/ml). Cultivation was carried out at four temperatures—45°C, 50°C, 55°C, and 60°C—for 25–30 days. Representative Petri dish images from water and soil-derived isolates are shown in Figure 1.

Comparative reference studies support the presence of thermophilic actinomycetes in Turkish geothermal environments. Aksoy et al. (2012) successfully isolated 163 thermophilic actinomycete strains from 28 hot springs and screened them for extracellular protease activity on casein-

enriched media. In another investigation, 85 thermophilic bacterial strains from hot springs in Erzurum were subjected to ERIC-PCR fingerprinting and biochemical profiling; 29 were sequenced and identified as *Bacillus*, *Pseudomonas*, *Silanimonas*, *Thermomonas*, or *Thauera* (Oztaş & Gormez, 2020).

Figure 1.

Some Actinobacteria *Isolation Petri Images a) T1-Water Sample ISP 2 Agar, b) T1-Soil Sample Starch Casein Agar*



Following extended incubation, actinobacterial colonies were selected based on distinct morphological characteristics. These colonies were purified using the line-inoculation technique on ISP2 agar supplemented with cycloheximide (50 µg/mL). Plates were incubated at 45 °C for 20 days to promote the growth of thermophilic actinobacteria.

Each purified isolate was assigned a unique code, cultured in pure form, and preserved in sterile screw-cap tubes containing 25% glycerol. Stocks were stored at -20 °C for long-term preservation. In total, 21 isolates were successfully cryopreserved (Table 4).

Genomic DNA Isolation, 16S rRNA Gene Sequence Analysis and Phylogeny

Genomic DNA was extracted from 14 isolates exhibiting distinctive colony morphologies. Amplification of the 16S rRNA gene yielded ~1500 bp fragments, as confirmed by agarose gel electrophoresis.

Three primers (518F, 800R, and MG5F), including two universal primers, were used for sequencing. The sequences were assembled with ChromasPro 1.7.5 and aligned in FASTA format. Identification was carried out using EzBioCloud, which enabled comparison with closely related taxa.

The results revealed that 8 of the isolates belonged to the genus *Streptomyces*, 4 were affiliated with *Nocardia*, while the remaining 2 were identified as *Actinomadura* and *Pseudonocardia*, respectively (Table 5; Figure 2). The nucleotide sequences were submitted to GenBank, and accession numbers are listed in Table 5.

Table 4.

List of the Isolated Microorganisms

	Organism no	Localities	Temperature (°C)	Dilution	Media name
1	TA01	T1-Soil	55 °C	10^{-4}	Yeast Extract-Malt Extract Agar-ISP2
2	TA07	T1-Soil	55 °C	10^{-3}	Bennett's Agar
3	TA13	T1-Soil	60°C	10^{-3}	Starch-Casein Agar
4	TA14	T1-Soil	60°C	10^{-3}	Starch-Casein Agar
5	TA17	T1-Soil	45°C	10^{-3}	Bennett's Agar
6	TA20	T1-Soil	45°C	10^{-3}	Bennett's Agar
7	TA24	T1-Soil	45°C	10^{-3}	Bennett's Agar
8	TA27	T1-Soil	45°C	10^{-3}	Bennett's Agar
9	TA28	T1-Soil	45°C	10^{-4}	Starch-Casein Agar
10	TA29	T1-Soil	45°C	10^{-4}	Starch-Casein Agar
11	TA35	T1-Soil	45°C	10^{-4}	Starch-Casein Agar
12	TA39	T1-Soil	50 °C	10^{-2}	Bennett's Agar
13	TA40	T1-Soil	50 °C	10^{-2}	Bennett's Agar
14	TA41	T1-Water	45°C	10^{-1}	Yeast Extract-Malt Extract Agar-ISP2
15	TA42	T1-Water	45°C	10^{-1}	Bennett's Agar
16	TA43	T1-Water	45°C	10^{-2}	Starch-Casein Agar
17	TA44	T1-Water	45°C	10^{-2}	Starch-Casein Agar
18	TA47	T1-Soil	50°C	10^{-4}	Starch-Casein Agar
19	TA53	T1-Soil	45°C	10^{-3}	Starch-Casein Agar
20	TA54	T1-Soil	45°C	10^{-3}	Starch-Casein Agar
21	TA58	T1-Soil	45°C	10^{-3}	Starch-Casein Agar

Phylogenetic analysis was conducted using the Neighbor-Joining method (Saitou & Nei, 1987). According to earlier guidelines, a 16S rRNA gene similarity below 97% was once considered a cutoff for species novelty (Stackebrandt & Goebel, 1994); however, current recommendations suggest raising the threshold to 98.7–99% to improve taxonomic resolution (Stackebrandt & Ebers, 2006).

Isolate TA39 displayed the lowest similarity (98.62%) to *Streptomyces thermoviolaceus subsp. thermoviolaceus* DSM 40443^T, differing at 20 nucleotide positions across 1450 aligned bases, indicating its potential novelty. Meanwhile, TA20 and TA58 showed 99.72% similarity to *Streptomyces albogriseolus* NRRL B-1305^T, and TA35 was closely related to the same species (99.59%). TA24, TA28, and TA29 aligned with *Streptomyces nigra* 452^T (99.45%). TA27 was similar to *Streptomyces thermocarboxydus* DSM 44293^T (99.86%).

Table 5.
Phylogenetic Similarity of the Test Organisms Belonging to the Class Actinobacteria *with the Closest Type Species According to 16S rRNA Sequence Results*

	No Strain Code	Genbank No	Closest Type	Similarity-Nucleotide Difference %
1.	TA20	PV490047	*Streptomyces albogriseolus* NRRL B-1305^T	99.72 % - 4/1449
2.	TA24	PV490049	*Streptomyces nigra* 452^T	99.45 % - 8/1448
3.	TA27	PV490134	*Streptomyces thermocarboxydus* DSM 44293^T	99.86 %- 2/1449
4.	TA28	PV490135	*Streptomyces nigra* 452^T	99.45 % - 8/1448
5.	TA29	PV490136	*Streptomyces nigra* 452^T	99.45 %- 8/1448
6.	TA35	PV490147	*Streptomyces albogriseolus* NRRL B-1305^T	99.59 %- 6/1449
7.	TA39	PV490148	*Streptomyces thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T	98.62 % - 20/1450
8.	TA41	PV490270	*Nocardia farcinica* NCTC 11134^T	100 %- 0/1441
9.	TA42	PV495233	*Nocardia farcinica* NCTC 11134^T	100 % - 0/1441
10.	TA43	PV523904	*Nocardia farcinica* NCTC 11134^T	100 % - 0/1441
11.	TA44	PV523905	*Nocardia farcinica* NCTC 11134^T	100 % - 0/1441
12.	TA47	PV533749	*Actinomadura sputi* IMMIB L-889^T	99.24 %- 11/1452
13.	TA54	PV533753	*Pseudonocardia adelaidensis* EUM 221^T	98.80 % –17/1412
14.	TA58	PV533764	*Streptomyces albogriseolus* NRRL B-1305^T	99.72 %- 4/1449

Other isolates included TA41–TA44, which showed complete identity (100%) with *Nocardia farcinica* NCTC 11134^T, TA47 with 99.24% similarity to *Actinomadura sputi* IMMIB L-889^T, and TA54 with 98.80% similarity to *Pseudonocardia adelaidensis* EUM 221^T, differing by 17 nucleotides over 1412 positions, suggesting it may also represent a novel species.

Investigation of Amylase and Protease Production Abilities

After 7 days of incubation on Starch agar, the amylase activity of each isolate was assessed by flooding the plates with 1% Lugol's iodine solution. The development of clear zones around the colonies against a dark blue background confirmed the hydrolysis of starch and hence, amylase activity (Figure 3, Table 6). Seven isolates showed positive results for amylase production.

Figure 2.
The dendrogram represents the phylogenetic relationships among all test isolates, constructed using the Neighbor-Joining algorithm based on 16S rRNA gene sequences. Bootstrap values are indicated at the branching points that show strong support, with values above 50% demonstrating statistical confidence in those branches. This tree helps visualize the evolutionary connections between the isolates, with closely related species grouped together. Nucleotide position change is 0.01

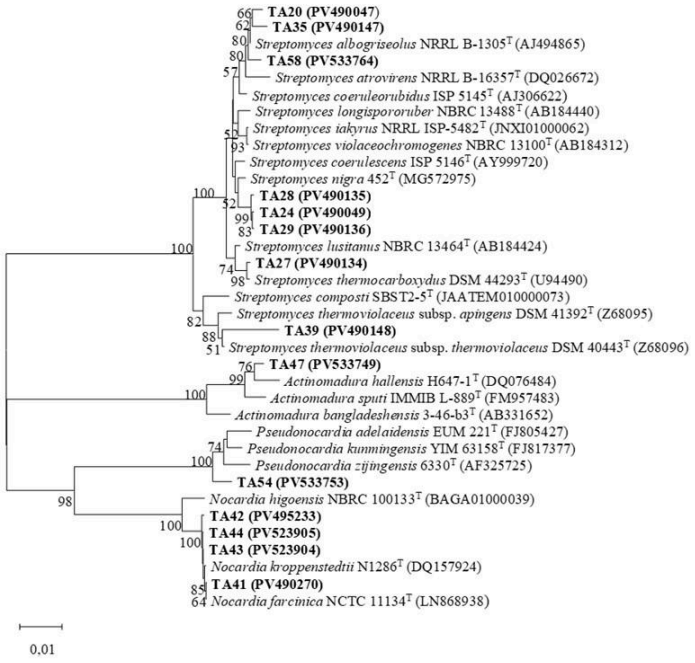
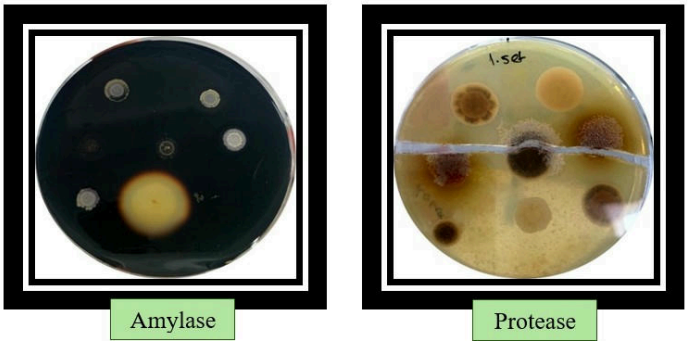


Figure 3.
Some Petri Images Showing Amylase and Protease Production Capacity



Proteolytic activity was assessed on casein-containing agar. Following 7 days of incubation, the appearance of transparent halos around the colonies indicated casein hydrolysis, a marker of protease production. Only one isolate demonstrated protease activity (Figure 3, Table 6).

Remarkably, a single *Streptomyces* strain showed both amylolytic and proteolytic capabilities, highlighting its

potential biotechnological relevance in applications requiring multifunctional enzymes.

Table 6.

Determination of Amylase and Protease Activities of the Isolates

No	Strain Code	Amylase Activity	Protease Activity
1	TA20	+	-
2	TA24	-	-
3	TA27	-	-
4	TA28	-	-
5	TA29	-	-
6	TA35	+	-
7	TA39	+	+
8	TA41	-	-
9	TA42	+	-
10	TA43	+	-
11	TA44	+	-
12	TA47	-	-
13	TA54	+	-
14	TA58	-	-

CONCLUSIONS

In this research, thermophilic actinobacteria were successfully isolated from the Terziköy Thermal Spring in Amasya, Türkiye, and their phylogenetic diversity and enzymatic properties were systematically evaluated. Based on 16S rRNA gene sequence analysis, most isolates were classified within the genus *Streptomyces*, a taxon widely known for its ability to produce antibiotics and industrial enzymes. Additionally, isolates related to *Nocardia*, *Actinomadura*, and *Pseudonocardia* were also identified, indicating that the thermal spring harbors a phylogenetically diverse actinobacterial community.

The enzyme screening experiments revealed that a considerable portion of the isolates exhibited amylase production. However, only a single isolate demonstrated protease activity. Notably, one *Streptomyces* strain showed dual enzymatic capabilities—amylase and protease—suggesting its promising potential for use in biotechnological applications requiring multifunctional enzyme producers.

These results underscore the ecological and biotechnological value of geothermal habitats, which serve as reservoirs of microbial diversity and novel enzyme sources. The adaptation of these actinobacteria to high-temperature environments equips them with the ability to produce thermostable enzymes suitable for industrial use, particularly under harsh processing conditions.

Future work guided by a polyphasic taxonomy approach—including whole-genome sequencing, DNA-DNA hybridization, and comprehensive phenotypic and chemotaxonomic analyses—will be essential for formal classification of novel taxa. In particular, isolates TA39 (*Streptomyces*) and TA54 (*Pseudonocardia*), which exhibited lower sequence similarities to known type strains, are strong candidates for description as novel species.

In conclusion, the actinobacteria isolated from Terziköy Thermal Spring represent valuable microbial resources with significant potential for enzyme production and natural product discovery. Exploration of such extreme environments is likely to contribute to the discovery of new strains with unique metabolic profiles, opening up new avenues for industrial and pharmaceutical biotechnology.

Acknowledgements The authors would like to express their sincere gratitude to the Hitit University Scientific Research Projects Coordination Department for providing financial support.

Ethics committee approval Ethics committee approval was not required for this study. All authors declare that no experiments involving human or animal subjects were conducted.

Peer Review Externally peer-reviewed.

Conflict of Interest The author have no conflict of interest to declare.

Grant Support This study was supported by Hitit University Scientific Research Projects Coordination Department with project number ODMYO19001.23.002.

Author Details

Demet Tatar

[1] Hitit University, Osmancık Ömer Derindere Vocational School, Department of Medical Services and Techniques, Çorum, Türkiye

0000-0002-9317-3263 demettatar@hitit.edu.tr

Aysel Veysioğlu

[2] Sinop University, Vocational School of Health Services, Department of Medical Services and Techniques, Sinop, Türkiye

0000-0002-1406-5513

Ali Tokatlı

[3] Technology Transfer Office, Çankırı Karatekin University, Çankırı, Türkiye

[4] Ondokuz Mayis University, Faculty of Science, Department of Biology, Samsun, Türkiye

0000-0002-7559-8882

Hünkar Avni Duyar

[5] Sinop University, Fisheries Faculty, Department of Fishing and Processing Technology, Sinop, Türkiye

0000-0002-2560-5407

References

Aksoy, S. Ç., Uzel, A. & Hameş Kocabaş, E. E. (2012). Extracellular serine proteases produced by Thermoactinomyces strains from hot springs and soils of West Anatolia. *Ann Microbiol*, 62, 483–492. https://doi.org/10.1007/s13213-011-0280-z

Amasya İl Kültür ve Turizm Müdürlüğü (2025, July 31), https://amasya.ktb.gov.tr/TR-243587/terzikoy-termal.html

Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot*, *58*(4), 1–26. https://doi.org/10.1038/ja.2005.1

Buchholz-Cleven, B. E. E., Rattunde, B., & Straub, K. L. (1997). Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. *Systematic and Applied Microbiology*, *20*, 301–309. https://doi.org/10.1016/S0723-2020(97)80077-X

Busarakam, K., Bull, A. T., Trujillo, M. E., Riesco, R., Sangal, V., van Wezel, G. P., & Goodfellow, M. (2016). *Modestobacter caceresii* sp. nov., novel actinobacteria with an insight into their adaptive mechanisms for survival in extreme hyper-arid Atacama Desert soils. *Syst Appl Microbiol*, *39*(4), 243–251. https://doi.org/10.1016/j.syapm.2016.03.007

Challis, G. L., & Hopwood, D. A. (2003). Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc Natl Acad Sci U S A100*, (Suppl 2), 14555–14561. https://doi.org/10.1073/pnas.1934677100

Chun, J. (1995). Computer Assisted Classification and Identification of Actinomycetes. PhD thesis. Department of Microbiology, University of Newcastle, Newcastle upon Tyne, UK.

Elbendary, A. A., Hessain, A. M., El-Hariri, M. D., Seida, A. A., Moussa, I. M., Mubarak, A. S., Kabli, S. A., Hemeg, H.A. & El Jakee, J.K. (2018). Isolation of antimicrobial producing Actinobacteria from soil samples. *Saudi J Biol Sci*, *25*(1), 44–46. https://doi.org/10.1016/j.sjbs.2017.05.003

Ertaş, H. B., Kiliç, A., Özbey, G., & Muz, A. (2005). Isolation of *Arcanobacterium (Actinomyces) pyogenes* from abscessed cattle kidney and identification by PCR. *Turk J Vet Anim Sci*, *29*(2), 455–459. https://journals.tubitak.gov.tr/veterinary/vol29/iss2/39

Felsenstein, J. (1985). Confidence limits on phylogeny: An appropriate use of the bootstrap. *Evolution*, *39*, 783–791. https://doi.org/10.2307/2408678

Fossi, B.T., Tavea, F., Jiwoua, C., & Ndjouenke, R. (2009). Screening and phenotypic characterization of thermostable amylases producing yeasts and bacteria strains from some Cameroonian soils. *African Journal of Microbiology Research*, *3*(9), 504–514.

Goodfellow, M. & Williams, S. T. (1983). Ecology of Actinomycetes. *Annu Rev Microbiol,* 37, 189–216. https://doi.org/10.1146/annurev.mi.37.100183.001201

Gorrab, A., Ouertani, R., Souii, A., Kallel, F., Masmoudi, A. S., Cherif, A., & Neifar, M. (2024). A comprehensive review on the properties and applications of extremozymes from extremophilic actinobacteria. *MOJ Applied Bionics and Biomechanics, 8(1),* 76-85. https://doi.org/10.15406/mojabb.2024.08.00213

Jones, K. L. (1949). Fresh isolates of Actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. Journal of Bacteriology, 57, 141–145. https://doi.org/10.1128/jb.57.2.141-145

Könönen, E., & Wade, W. G. (2015). Actinomyces and related organisms in human infections. *Clin Microbiol Rev*, *28*(2):419–442. https://doi.org/10.1128/CMR.00100-14

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). Molecular evolutionary genetics analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, *41*(12), 1–9. https://doi.org/10.1093/molbev/msae263

Küster, E., & Williams, S. T. (1964). Selection of media for isolation of streptomycetes. *Nature*, 202, 928–929. https://doi.org/10.1038/202928a0

Lacey, J. (1988). Actinomycetes as biodeteriogenes pollutants of the environment. In: Actinomycetes in Biotechnology, Goodfellow, M., Williams, S., Mordarski, M., Eds. Academic Press. San Diego: 359–417.

Lerat, S., Simao-Beaunoir, A. M., & Beaulieu, C. (2009). Genetic and physiological determinants of *Streptomyces scabies* pathogenicity. *Molecular Plant Pathology.* 1 0, 579–585. https://doi.org/10.1111/j.1364-3703.2009.00561.x

Ma, G., Xia, Z., & Wu, S. (2009). Inhibiting effect of seven marine actinomycete strains against vegetable pathogenic microorganisms. *Crops*, *5*, 3–9.

Medjemadj, M., Escuder-Rodríguez, J. J., Boudemagh, A., & González-Siso, M. I. (2020). Actinobacteria isolated from Algerian hot spring waters: A potential source of important enzymes. *Eco. Env. & Cons, 26*(3), 1145-1157.

Mohamedin, A. H. (1999). Isolation, identification and some cultural conditions of a protease-producing thermophilic *Streptomyces* strain grown on chicken feather as a substrate. *International Biodeterioration and Biodegradation*, 43, 13–21. https://doi.org/10.1016/S0964-8305(98)00061-4

Oztas Gulmus, E., & Gormez, A. (2020). Identification and characterization of novel thermophilic bacteria from hot springs, Erzurum, Turkey. *Curr Microbiol*, *77*(6), 979–987. https://doi.org/10.1007/s00284-020-01880-0

Pathom-Aree, W., Stach, J. E., Ward, A. C., Horikoshi, K., Bull, A. T., & Goodfellow, M. (2006). Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. *Extremophiles*, *10*(3), 181–189. https://doi.org/10.1007/s00792-005-0482-z

Rego, A., Raio, F., Martins, T. P., Ribeiro, H., Sousa, A. G. G., Séneca, J., Baptista, M. S., Lee, C. K., Cary, S. C., Ramos, V., Carvalho, M. F., Leão, P. N., & Magalhães, C. (2019). *Actinobacteria* and *Cyanobacteria* diversity in terrestrial Antarctic microenvironments evaluated by culture-dependent and independent methods. *Front Microbiol*, *10*, 1018. https://doi.org/10.3389/fmicb.2019.01018

Saitou, N., & Nei, M. (1987). The neighbour-joining method: A new method for constructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16, 313–340. https://doi.org/10.1099/00207713-16-3-313

Shivlata, L., & Satyanarayana, T. (2015). Thermophilic and alkaliphilic *Actinobacteria*: Biology and potential applications. *Frontiers in Microbiology*, 6, 1014. https://doi.org/10.3389/fmicb.2015.01014

Shomali, B. A., & Danish-Daniel, M. (2024). Review on bioprospecting of thermophilic enzymes from hot springs via omics approaches. *AACL Bioflux, 17*(5), 2156-2174. Retrieved from https://bioflux.com.ro/docs/2024.2156-2174.pdf

Stackebrandt, E., & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today*, *33,* 152–155.

Stackebrandt, E., & Goebel, B. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, *44*(4), 846–849. https://doi.org/10.1099/00207713-44-4-846

Takahashi, Y., & Omura, S. (2003). Isolation of new actinomycete strains for the screening of new bioactive compounds. *J Gen Appl Microbiol, 49*(3), 141–154. https://doi.org/10.2323/jgam.49.141

Tang, H., Kambris, Z., Lemaitre, B., & Hashimoto, C. (2006). Two proteases defining a melanization cascade in the immune system of *Drosophila*. *J Biol Chem*, *281*(38), 28097-28104. https://doi.org/10.1074/jbc.M601642200

Veyisoglu, A., & Tatar, D. (2021). Diversity and antimicrobial activity of culturable actinobacteria isolated from the sediment of Sarıkum Lake. *Biotechnology & Biotechnological Equipment*, *35*(1), 1136–1146. https://doi.org/10.1080/13102818.2021.1952898

Veyisoglu, A., Tatar, D., Duyar, H. A., & Tokatli, A. (2024). Isolation, molecular characterization and determination of antagonistic properties of alkalitolerant *Streptomyces* members from Van lake-Çarpanak island soil. *Marine Science and Technology Bulletin*, *13*(3), 183–198. https://doi.org/10.33714/masteb.1522501

Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: A taxonomically united daltabansa of 16S rRNA and whole genome assemblies. *Int J Syst Evol Microbiol*, *67*(5), 1613–1617. https://doi.org/10.1099/ijsem.0.001755